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Synthesis of 1,7-annulated indoles and their applications in the studies of cyclin dependent kinase inhibitors ☆

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Abstract—The synthesis of a novel series of 1,7-annulated indolocarbazoles **2** and **16** is described. These compounds were found to be potent cyclin dependent kinase inhibitors with good antiproliferative activity against two human carcinoma cell lines. These inhibitors also arrested tumor cells at the G1 phase and inhibited pRb phosphorylation.

The cyclin dependent kinases (CDKs) are serine/threonine protein kinases that regulate the cell division cycle, apoptosis, transcription, and differentiation.[1] Specific CDKs operate in distinct phases of the cell cycle. In general, the activity of this family of kinases requires binding of a cyclin regulatory subunit. Frequent genetic mutation of CDK substrates and deregulation of CDKs in various diseases have stimulated an intensive search for CDK inhibitors. In particular, the D type cyclins, D1, D2, and D3, associate with CDK 4 and 6 are believed to play a critical role early in the G1 phase of the cell cycle and their activities are frequently deregulated in many type of cancers.[2] These complexes phosphorylate the retinoblastoma protein and inactivate its ability to act as a transcriptional repressor in a complex with E2F.[3a] Thus, inhibitors of these cyclin dependent kinases that stop uncontrolled tumor cell growth represent attractive new therapeutic agents for the treatment of cancer.[3]

A variety of structural scaffolds have been disclosed as CDK inhibitors,[3] including three compounds (flavopiridol,[4] UCN-01,[5] and roscovitine[6]) in clinical development as anticancer therapeutics. However, the majority of them have been found to inhibit CDK1 and/or CDK2 very potently,[7] and relatively fewer selective D1/CDK4 inhibitors have been discovered.[8,9] During our research program to discover selective D1/CDK4 inhibitors, we found that simple indolocarbazole analogs (**1**, Fig. 1) possess potent and selective inhibitory activity against D1/CDK4.[9a] Because of the flat and hydrophobic structural properties, this class of compounds has very poor aqueous solubility. To optimize the pharmacokinetic properties of these CDK4 inhibitors, we explored novel analogs of these indolocarbazoles by introducing a 1,7-annulated ring in one of the indoles represented by compound **2**.[9c] We hypothesized that the substitution on the annulated ring can decrease the planarity of the overall molecule, thus improving the compounds' solubility.

Initially, 1,7-annulated indole derivatives **3** were selected for rapid SAR expansion by incorporating a

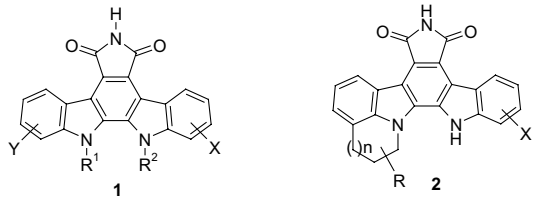

Figure 1.

Keywords: 1,7-Annulated indole; D1/CDK4 inhibitor; Cancer.
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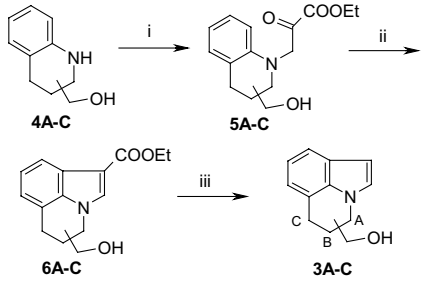
hydroxymethyl group as a chemical handle for further elaboration. The 1,7-annulated indole **3** was synthesized as shown in Scheme 1. Indole ring closure of the alkylation product **5** of tetrahydroquinoline derivatives (**4A**–**C**) with ethyl bromopyruvate in the presence of $MgCl_2$ gave compound **6**.[10] Subsequent hydrolysis of **6** under basic conditions and decarboxylation provided **3**.

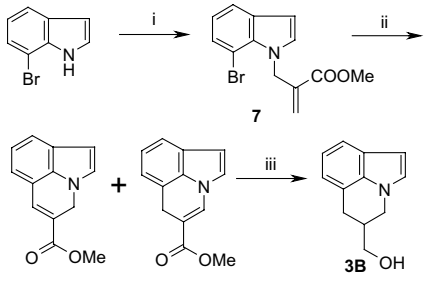
Alternatively, **3B** can be derived from 7-bromoindole (Scheme 2). Alkylation of the indole nitrogen gave compound **7**, which underwent an intramolecular Heck reaction to form the 1,7-annulated ring. Subsequent hydrogenation and $LiAlH_4$ reduction gave compound **3B** in good yield.

A readily scalable method was developed for compound **3B** using 7-formylindole as starting material as illustrated in Scheme 3. N-Alkylation of 7-formylindole with methyl 3-bromopropionate followed by cyclization in the presence of cesium carbonate gave unsaturated cyclic product **8B** in one pot,[11] that was hydrogenated to afford **9B**. $LiAlH_4$ reduction of **9B** gave compound **3B** in good yield. This method was also applicable to seven-membered annulated indole **3D** using methyl 4-bromobutyrate as an alkylating agent.

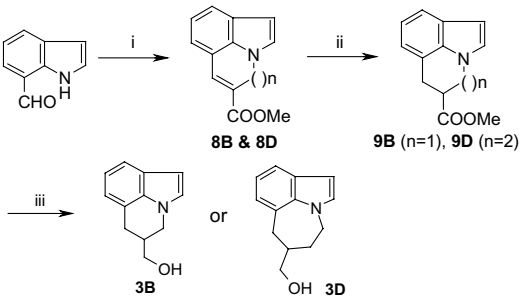
Indolocarbazole **13** was prepared from the cyclization of the corresponding bisindolyl maleimide **12** as shown in Scheme 4. The key cyclization precursor bisindolyl maleimide **12** was synthesized in good yield via KO^{t}Bu promoted condensation of an appropriately substituted indolyl acetamide **11** and an indolyl 3-glyoxylate **10**



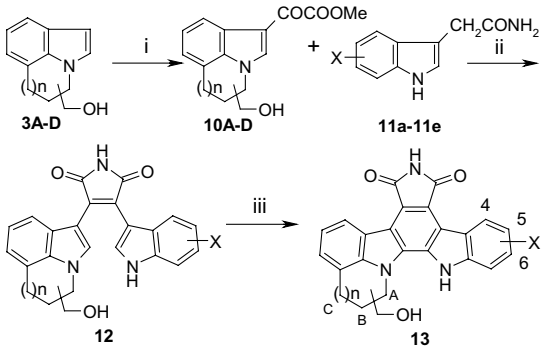
Scheme 1. Reagents and conditions: (i) $BrCH_2COCOOEt$, THF, rt; (ii) $MgCl_2$, $CH_3OCH_2CH_2OH$, 125 °C; (iii) (a) NaOH, EtOH–H_2O; (b) copper chromite, quinoline, 185 °C.



Scheme 2. Reagents and conditions: (i) $BrCH_2C(CH_2)COOCH_3$, KOH, DMSO, 58%; (ii) $Pd(OAc)_2$, $NaHCO_3$, DMF, *n*-Bu_4NCl, 96%; (iii) (a) H_2, Pd/C, EtOAc; (b) $LiAlH_4$, THF, 41%.



Scheme 3. Reagents and conditions: (i) $BrCH_2(CH_2)_nCOOCH_3$, Cs_2CO_3, DMF, 80 °C, 29–50%; (ii) H_2, Pd/C, EtOAc, 100%; (iii) $LiAlH_4$, THF, 90–95%.



Scheme 4. Reagents and conditions: (i) (a) $(COCl)_2$, ether, −78 °C; (b) NaOMe, MeOH, 60–90%; (ii) (a) KO^{t}Bu, THF; (b) concd HCl, 60–90%; (iii) $Pd(OAc)_2$, AcOH, Δ, or DDQ, solvent, *p*-TsOH reflux, or I_2, benzene, hν.

based on Faul's procedure.[12] Indolyl 3-glyoxylate can be obtained in good yield from corresponding annulated indole **3**. Oxidation of **12** to indolocarbazole **13** was performed in 50–80% yield using $Pd(OAc)_2$ in acetic acid. Alternatively, the oxidative cyclization could be carried out under other conditions previously reported for this transformation (hν with or without I_2 or DDQ with or without *p*-TsOH).[9a]

D1/CDK4 enzyme inhibitory activities of indolocarbazole **13** were determined by measuring the phosphorylation of Rb protein.[9a,13] Compounds were also tested in other kinase inhibitory assays by measuring the phosphorylation of the corresponding substrates (e.g., E-CDK2 using Rb^{ING} as substrate, PKA using histone as substrate). Staurosporine, the well-known kinase inhibitor, was used as a standard compound for the assays. In addition, effects on cell proliferation in vitro were determined in two human carcinoma cell lines, HCT-116 (colon) and NCI-460 (lung).[14] Data were summarized in Table 1. Indolocarbazoles **13Aa**, **13Ba**, and **13Ca** exemplified the effect of hydroxymethyl at varying positions of the annulated ring. All three compounds were potent inhibitors against cyclinD1/CDK4 and were effective antiproliferative agents. Cell cycle effects in HCT-116 cells were then examined by flow cytometry.[9a,15] Compound **13Ba** showed a more significant G1 cell cycle arrest than compounds **13Aa** and

Table 1. Kinase inhibitory activity (IC_{50}, μM) against D1-CDK4, E-CDK2, and PKA, antiproliferative activity (IC_{50}, μM), and cell cycle G1 arrest (fold increase over control) in HCT-116 (colon) cell line

Compd	Position of CH_2OH	X	*n*	CDK4 (Rb^{21})	CDK2 (Rb^{ING})	PKA	HCT-116	NCI-H460	G1 arrest[a]		Solubility (mg/mL)[e]
									@1×IC_{50}	@3×IC_{50}	
13Aa	A	H	1	0.018	0.219	0.858	0.92	0.82	1.32	1.51	0.57
13Ba	B	H	1	0.071	0.217	>2.0	0.91	0.96	2.04	1.95	0.12
13Ca	C	H	1	0.031	—[b]	>2.0	0.37	0.54	1.17	1.44	—[b]
13Ab	A	6-F	1	0.002	0.079	3.02	0.82	0.26	1.70	2.12	0.62
13Bb	B	6-F	1	0.022	0.141	>2.0	0.36	0.43	1.67	2.32	0.89
13Bc	B	6-Br	1	0.010	>2.0	>2.0	0.50	0.36	1.60	—[b]	0.83
13Bd	B	6-CF_3	1	0.023	—[b]	>2.0	1.25	0.69	1.34	1.47	4.81
13Be	B	6-OMe	1	0.015	0.284	>2.0	1.83	1.96	1.80	2.00	0.49
13Da	B	H	2	0.126[c]	—[b]	—[b]	0.54	0.33	1.80	1.90	1.07
13Af	A	—[d]	—[d]	0.175	—[b]	>2.0	1.12	0.54	1.17	1.32	2.78
13Bf	B	—[d]	—[d]	0.072	>2.0	>2.0	0.94	0.74	1.14	1.64	—[b]
13Cf	C	—[d]	—[d]	0.210	—[b]	>2.0	1.25	1.72	1.20	1.30	—[b]
13Bg	B	—[d]	—[d]	0.084	0.650	—[b]	0.68	0.21	1.13	2.25	—[b]
16a	—[d]	—[d]	—[d]	0.064	—[b]	>20	3.27	5.65	—[b]	—[b]	—[b]
16b	—[d]	—[d]	—[d]	0.008	—[b]	0.383	1.73	5.15	—[b]	—[b]	—[b]
16c	—[d]	—[d]	—[d]	0.898[c]	—[b]	—[b]	0.77	4.75	1.0	1.17	—[b]
16d	—[d]	—[d]	—[d]	0.671[c]	—[b]	—[b]	>10	>10	—[b]	—[b]	—[b]
16e	—[d]	—[d]	—[d]	>2.0[c]	—[b]	—[b]	>10	>10	—[b]	—[b]	—[b]

[a] After 24 h incubation, measured as the fold increase of G1 population over the control.
[b] Not tested.
[c] Rb^{ING} as substrate; see Refs. 9a,12.
[d] Not applicable.
[e] Solubility measured in 10% solutol in EtOH.

13Ca in this assay. Further SAR study was focused on the derivatization of compound **13Ba** with the hydroxymethyl in the central carbon position B as shown in Scheme 4. The evaluation of the substitution on the nonannulated indole ring was focused on the 6-position based on our early finding that substitution on this position was most favorable for D1/CDK4.[9a] The fluoro (**13Bb**), bromo (**13Bc**), trifluoro methyl (**13Bd**), and methoxyl (**13Be**) groups resulted in very potent inhibitors of cyclin D1/CDK4 with selectivity against cyclinE/CDK2 and PKA. In addition, they also have antiproliferative effect in the HCT-116 colon carcinoma cell line and NCI-460 lung carcinoma cell line with activities ranging between 0.36 and 1.98 μM. All these compounds arrest cells in the G1 phase in HCT-116 cells with an exception of trifluoromethyl analog **13Bd**, which only had moderate effect on G1 arrest.

The ring size did not appear to affect the activity; the seven-membered analog **13Da** was equally potent against cyclin D1/CDK4 compared to six-membered compound **13Ba**.

We also studied the replacement of the indole moiety with other aryl/heteroaryl rings. Figure 2 exemplifies the corresponding naphthyl and reversed indole analogs. All of these compounds exhibited potent inhibitory activity against D1/CDK4 with good antiproliferative cell activity in HCT-116 and NCI-460. Similar to the previous finding, naphthyl analog **13Bf**, with the hydroxymethyl in the central carbon position **B**, gave a better overall profile.

Based on the finding that the hydroxymethyl on the central carbon **B** position was well tolerated, we turned

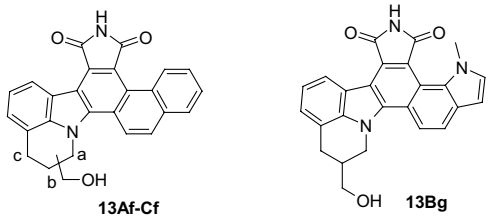


Figure 2.

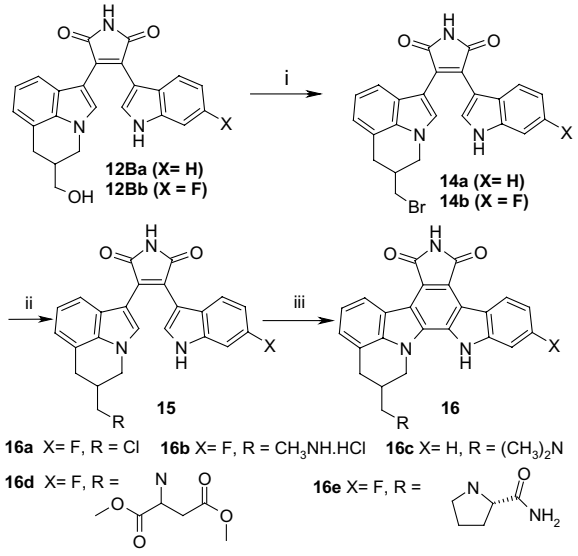
our attention to utilize this group as a chemical handle to introduce more aqueous soluble groups to improve the biopharmaceutical properties of the indolocarbazole series. The hydroxyl group on **12** was converted to bromo analog **14** using bromine and triphenyl phosphite. Subjecting bromide **14** to a variety of amines resulted in analogs **15** with improved aqueous solubility. Oxidative cyclization of maleimide **15** under previously described conditions gave the desired carbazole compound **16** (Scheme 5). The methylamine analog **16b** was a very potent inhibitor against D1/CDK4 with an IC_{50} of 8 nM, however the cellular activity in HCT-116 and NCI-460 was moderate. This may be due to poor cell penetration of the amine analog. Further increase in the size of the amine group led to less potent compounds (**16c**, **16d**, and **16e**).

It is clear that CDK4 plays a critical role in the G1-S transition of the cell cycle by phosphorylating Rb. It is also known that the Ser-780 residue on Rb is specifically phosphorylated by CDK4.[16] Thus, inhibition of cellular CDK4 activity will result in the inhibition of Rb phosphorylation on Ser-780 and cell cycle arrest in the G1 phase. So we evaluated the effects of the compounds on

Table 2. pRb phosphorylation inhibition by D1/CDK4 inhibitors[a]

	Compounds						
	13Aa	**13Ba**	**13Ca**	**13Bb**	**13Bc**	**13Be**	**13Da**
HCT-116 IC_{50} (μM)	0.92	0.91	0.37	0.36	0.5	0.83	0.54
%pRb inhibition @ IC_{50}	53	21	0	25	56	69	71
%pRb inhibition @2 IC_{50}	65	55	23	53	74	77	85

[a] Colon carcinoma cells (HCT-116) cells treated with compounds at 1× and 2× antiproliferation IC_{50} concentration for 24 h.



Scheme 5. Reagents and conditions: (i) $(PhO)_3P$, Br_2, methylene chloride; (ii) amine (R), NMP, rt; (iii) $Pd(OAc)_2$, AcOH, Δ, or DDQ, solvent, *p*-TsOH reflux, or I_2, benzene, h*v*.

CDK4 activity by measuring phospho-Rb^{S780} levels using a phospho-specific antibody. Colon carcinoma cells (HCT-116) cells were treated with compounds at 1× and 2× antiproliferation IC_{50} concentration for 24 h, followed by the Western Blot analysis. As shown in Table 2, these compounds potently inhibited phosphorylation of Rb^{S780} demonstrating that they inhibit cellular CDK4 activity. These specific observations on the G1 cell cycle arrest and inhibition of phosphorylation of serine 780 on pRb indicates that these indolocarbazoles are potent CDK4 inhibitors.

In conclusion, we have described the synthetic approaches for a class of indolocarbazoles with a substituted 1,7-annulated ring from readily available starting materials. These compounds were found to be potent D1/CDK4 inhibitors and antiproliferative agents in HCT-116 and NCI-460 cell lines. In addition, the specific G1 cell arrest and selective inhibition of phosphorylation of serine 780 on pRb are consistent with the in vitro D1/CDK4 inhibitory activity.

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